Survival and Growth of Pine Seedlings with Pisolithus *Ectomycorrhizae after Two Years on Reforestation Sites in North Carolina and Florida*

DONALD H. MARX
WILLIAM C. BRYAN
CHARLES E. CORDELL

ABSTRACT. Two-year field data indicated that ectomycorrhizae formed by *Pisolithus tinctorius* increase survival and growth of five southern pine species on routine reforestation sites. The seedlings with ectomycorrhizae of *Pisolithus* were grown earlier in nurseries using either mycelial or basidiospore inoculum to form the ectomycorrhizae. Control seedlings had ectomycorrhizae formed by fungi (mainly *Thelephora terrestris*) that occurred naturally in the nurseries. Species that had significantly greater survival after two years with *Pisolithus* ectomycorrhizae than with natural nursery ectomycorrhizae were loblolly pine (*Pinus taeda* L.) and eastern white pine (*P. strobus* L.) on one site and Virginia pine (*P. virginiana* Mill.) on two sites in North Carolina; and slash pine (*P. elliottii* Englem. var. *elliottii*) and sand pine (*P. clausa* var. *immuginata* Ward.) on two sites in Florida. A plot volume index (PVI) was developed for measurement of total growth response of seedlings. Significant increases in PVI due to *Pisolithus* ectomycorrhizae over natural ectomycorrhizae were observed; they varied from 25 percent for loblolly pine in North Carolina to 450 percent for sand pine in Florida and eastern white pine in North Carolina. On fertilized (90 kg/ha of N and P) plots in Florida, *P. tinctorius* did not increase the PVI over control slash pines, but this fungus increased PVI of seedlings by 175 percent on the nonfertilized plots. *P. tinctorius* persisted on roots of seedlings and produced many basidiocarps near seedlings of all pine species, especially on the poorer sites. Indigenous symbiotic fungi also formed significant quantities of ectomycorrhizae, particularly on the better sites. These results indicate that *P. tinctorius* ectomycorrhizae can persist and increase survival and growth of southern pines better than natural ectomycorrhizae on a variety of reforestation sites. FOREST SCI. 23:363–373.

ADDITIONAL KEY WORDS. *Cenococcum graniforme, Rhizopogon* sp., seedling growth parameters, fungal symbiont.

WORKERS in various parts of the world have reported growth increases of tree seedlings following inoculation of soil with pure cultures of ectomycorrhizal fungi in greenhouse or similar environments (Fassi and Fontana 1969, Theodorou and Bowen 1970, Theodorou 1971, Vozzo and Hacskaylo 1971, Lamb and Richards 1974). Only a few trials, however, have been conducted in conventional or simulated nurseries (Takacs 1967; Moser 1958, 1959; Göbl 1975; Donald 1975; Marx and Bryan 1975) or in production of container stock for reforestation (Göbl 1974; Marx and Barnett 1974). These trials showed that the use of pure cultures of ecto-

---

The authors are Director and Plant Pathologist, Institute for Mycorrhizal Research and Development, Southeastern Forest Experiment Station, Athens, Georgia 30602; and Nursery, Seed Orchard and Regeneration Disease Specialist, Forest Insect and Disease Management, Southeastern Area, State and Private Forestry, Asheville, North Carolina 28803, respectively.
Manuscript received January 25, 1977.

mycorrhizal fungi can improve ectomycorrhizal development on seedlings and increase growth of seedlings after outplanting. In the United States, however, there are no data on the performance of tree seedlings ectomycorrhizal with specific fungi under field conditions normally encountered in reforestation. Performance of trees has been reported (Marx 1976) with specific mycorrhizae in reclamation efforts on adverse planting sites.

Recently, *Pisolithus tinctorius* (Pers.) Coker and Couch was successfully introduced into fumigated soil in tree nurseries (Marx and others 1976), where it formed abundant ectomycorrhizae and stimulated pine seedling growth. The research reported here was a comparison of field performance of seedlings of five species of southern pines having ectomycorrhizae formed by *P. tinctorius* to those with ectomycorrhizae formed by fungi that naturally occurred in the nurseries on five reforestation sites.

Materials and Methods

Pisolithus tinctorius was introduced as mycelium in a vermiculite medium and as basidiospores into fumigated nursery soils, and ectomycorrhizae were formed on various pine species (Marx and others 1976). In the Florida nursery, abundant ectomycorrhizae were formed by *P. tinctorius*, particularly from vegetative mycelial inoculum; but the growth of the seedlings was not significantly greater than that of control seedlings with natural ectomycorrhizae. In the North Carolina nursery, however, abundant ectomycorrhizae were formed by both types of *Pisolithus* inocula, which induced a doubling of seedling growth in comparison to noninoculated seedlings.

Control and *Pisolithus*-inoculated seedlings of all tree species not used in the previous study (Marx and others 1976) were graded to uniform heights and stem diameters ($\pm$ 10 percent) between and within mycorrhizal treatments within 2 days after lifting. *Thelephora terrestris* Ehrh. ex Fr. and other naturally occurring fungi had formed ectomycorrhizae on the control seedlings. Before outplanting, the ectomycorrhizal development on graded seedlings approximated that on seedlings in the previous study (Marx and others 1976). Seedlings were stored at 5°C in moist peat moss in plastic-coated paper bags for 2 to 5 days prior to planting sites 1 through 4. Seedlings planted on site 5 were stored for nearly 3 months prior to planting.

Characteristics of the five outplanting sites and experimental designs were as follows.

Site 1 is in Burke County, North Carolina. The previous timber stand was mature, mixed pine-hardwood which was clearcut in May 1973. In June 1973, the herbicide 2,4,5-T was applied to control weeds and hardwood sprouts. Debris was windrowed and burned in November 1973. The site has less than 1 percent slope with 20 to 25 cm of friable topsoil. The soil, a clay loam, contained 5 percent organic matter, 17 ppm NO_3-N, and 11 ppm of K and had a pH of 4.3. Phosphorus, Ca, Mg, and Mn were not determined.[1]

The site was planted in December 1973 with seedlings grown at the Edwards State Nursery, Morganton, North Carolina. Seedling treatments were: loblolly pine (*Pinus taeda* L.) with ectomycorrhizae formed by *P. tinctorius* from vegetative mycelial inoculum (VMI), and from basidiospore inoculum (BI), and with ectomycorrhizae formed by *T. terrestris* and other naturally occurring fungi as the control (NI); Virginia pine (*P. virginiana* Mill.) with ectomycorrhizae formed by VMI,

[1] Analyses of soil samples were performed by the Soil Testing and Plant Analysis Laboratory, Cooperative Extension Service, University of Georgia, Athens 30602.

BI, and NI; and white pine (*P. strobus* L.) with ectomycorrhizae formed by VMI and NI. Sufficient white pine seedlings from the BI treatment were not available.

A split-plot design with 5 blocks was used. Each plot contained one row (subplot) of 20 seedlings of each pine species in an ectomycorrhizal treatment. The main plots were ectomycorrhizal treatments and the subplots were pine species. Rows were 2.4 cm apart and seedlings were planted by hand 1.2 m apart in each row. To minimize contamination between main plots (Marx 1976), they were separated by 4.8 m nonplanted strips. Blocks were separated by 10 m nonplanted strips.

Site 2 is approximately 500 m from Site 1 and the previous stand and site preparations were the same. The site slopes 12 to 15 percent west to east. On account of erosion, less than 4 cm of friable topsoil remains. The soil is a clay loam and has 3.1 percent organic matter, 11, 40, 73, 21, and 11 ppm of NO_3-N, K, Ca, Mg, and Mn, respectively. Soil pH was 4.8. Phosphorus was not determined.

The source of seedlings, time of planting, and the field design were identical to those on Site 1, except that only loblolly pines from the VMI, BI, and NI treatments and Virginia pines from the VMI and NI treatments were planted. Sufficient white pines from either ectomycorrhizal treatment or Virginia pines from the BI treatment were not available.

Site 3 is in a seed orchard near Brooksville, Florida. The previous ground cover was an unidentified leguminous plant which was disked into the soil prior to planting seedlings. The site is a ridge of well-drained, Arredonda fine sand which contains 1.2 percent organic matter, 2, 75, 27, 110, 22, and 2 ppm of NO_3-N, P, K, Ca, Mg, and Mn, respectively. Soil pH was 5.5.

The site was planted in January 1974 with seedlings from the Andrews Nursery, Chiefland, Florida. The field design was identical to that on Site 1. Seedling treatments were loblolly pine and slash pine (*P. elliottii* Englem. var. *elliottii*), each with VMI, BI, and NI; and sand pine (*P. clausa* var. *immuginata* Ward) with VMI and NI. Sand pine seedlings from the BI ectomycorrhizal treatment were not available.

Site 4, also near Brooksville, Florida, is a typical palmetto-flatwood site which supported a mature, natural slash pine stand before clearcutting in the summer of 1973. The site was burned, roll-chopped, and bedded (0.5 m high) on 3.5- to 4-m centers in the fall of 1973. The soil is a poorly drained, sandy loam with a brown organic hardpan (45 cm from surface soil) and a light grey subsoil. The soil contained 3.9 percent organic matter, 3, 23, 155, and 50 ppm of P, K, Ca, and Mg, respectively, and had a pH of 4.3. Nitrate-N and Mn were not determined.

The source of seedlings, time of planting, and seedling treatments were the same as at Site 3. Since this site was bedded on 3.5 to 4 m centers, it was not necessary to have nonplanted rows separating main plots. Otherwise, the field design was the same as Site 3.

Site 5, near Carrabelle, Florida, was clearcut of mature slash pine, prepared, and bedded on 3.2 m centers as on Site 4. The soil is a poorly drained, Rutledge sandy loam. It was not chemically analyzed, but Rutledge soils typically are low in all major elements, especially P.

The site was planted in April 1974 with slash pine seedlings obtained from the Andrews Nursery, Chiefland, Florida. The field design was random, with three replicate plots per treatment. The four treatments were slash pine with VMI and NI, with either no fertilizer or 90 kg/ha of N (as NH_4NO_3) plus 90 kg/ha of P (as triple phosphate) broadcast after planting. Each plot contained five rows of 10 seedlings, planted at 1 m intervals on the beds. Plots were separated by nonplanted strips of 3 m.

Height and stem collar diameters of all seedlings were measured at ground level in January after the first and second seasons. Evaluation of ectomycorrhizal devel-

opment and persistence of *Pisolithus* was also made at those times. After the first season, approximately 2 liters of soil, with roots, were removed with a shovel from the base of each of three randomly selected seedlings per subplot. The degree of ectomycorrhizal development was visually estimated without magnification on each site. The presence of cinnamon-brown ectomycorrhizae or hyphal strands of *Pisolithus*, as illustrated earlier (Marx and Bryan 1975), was considered as proof of the persistence of the fungus. The presence of basidiocarps of *Pisolithus* and other ectomycorrhizal fungi was also recorded.

After the second season, above-ground fresh weights of three seedlings per subplot were obtained from Sites 1 through 4. These seedlings were selected after determining the average height and root collar diameters of all the seedlings in a subplot. Then the smallest and largest seedlings were harvested; the third seedling was chosen such that the average height and root collar diameter of the three seedlings approximated (within 10 percent) the averages of the subplot. These seedlings were weighed on site immediately after cutting the stems at ground level. Where only three or fewer seedlings survived per subplot, all seedlings were harvested and weighed. Second-year data on ectomycorrhizal development were obtained by removing the majority of the roots of the harvested seedlings in each subplot.

Survival and growth data after two seasons were integrated into indices of volume and weight. Height $\times$ (root collar diameter)^2 was considered to be an indicator of seedling volume. Plot volume index (PVI) was determined by multiplying mean seedling volume by the number of surviving seedlings per subplot. Plot weight index (PWI) was obtained by multiplying the mean fresh top weight (gm) of seedlings by the number of surviving seedlings per subplot. Since fresh weight is widely used to compare treatment effects on growth, it and PWI were compared to seedling volume and PVI to assess the value of the latter two measurements as growth indices.

Analyses of variance were made on all data and differences among means were evaluated with Duncan's Multiple Range Test ($P = 0.05$).

Results

Trends in growth response to ectomycorrhizal treatment appeared in the first season and became statistically significant in the second season. Survival varied little between the first and second season. All pine species with a large amount of *Pisolithus* ectomycorrhizae at planting grew faster than control seedlings with natural ectomycorrhizae. Survival and growth of each species are reported for each site.

Site 1. Ectomycorrhizae of *P. tinctorius* did not significantly increase survival, height, or root collar diameter of loblolly or Virginia pines (Table 1). Loblolly pines with *Pisolithus* from VMI and BI treatments and Virginia pine from the VMI treatment, however, had greater fresh weight, seedling volume, PVI, and PWI than seedlings from the NI treatments. *Pisolithus* ectomycorrhizae significantly improved all aspects of white pine performance. Seedling volume, fresh weight, and plot indices were three to five times greater than white pine with natural ectomycorrhizae.

Pisolithus was found on roots of all seedlings of each pine species from the VMI treatment. After the first and second seasons, an average of 2 and 20 basidiocarps, respectively, were found in each loblolly pine subplot, 1 and 25 in Virginia pine, and 0 and 2 in white pine in the VMI treatment. Also, after the first and second seasons, over 80 percent of both loblolly and Virginia pine seedlings from the BI treatment had abundant *Pisolithus* ectomycorrhizae. Basidiocarps of *P. tinctorius* were not detected near seedlings in this treatment after the first year, but an average

TABLE 1. Survival and growth of loblolly, Virginia, and eastern white pine seedlings with Pisolithus tinctorius *or natural ectomycorrhizae after 2 years on Site 1 near Morganton, NC. Each value is the mean of 5 replicates each originally containing 20 seedlings.*[1]

Ectomycorrhizae from nursery formed by—	Survival (percent)	Height (cm)	Root collar dia (cm)	Seedling vol. (cm³)[2] / 100	PVI (cm³)[3] / 100	Top fresh wt (gm)	PWI (gm)[4] / 100
			LOBLOLLY PINE				
Pisolithus as vegetative inoculum (VMI)	93 a	85.1 a	2.06 a	3.61 a	67.2 a	433 a	80.5 a
Pisolithus as spore inoculum (BI)	91 a	86.1 a	2.11 a	3.83 a	69.7 a	460 a	83.7 a
Natural inoculum (NI)	92 a	79.1 a	1.91 a	2.89 b	53.2 b	358 b	65.9 b
			VIRGINIA PINE				
Pisolithus as vegetative inoculum	96 a	84.6 a	2.35 a	4.67 a	89.7 a	720 a	138.2 a
Pisolithus as spore inoculum	88 a	80.4 a	2.19 a	3.86 b	67.9 b	518 b	91.2 b
Natural inoculum	92 a	82.5 a	2.15 a	3.81 b	70.1 b	472 b	86.8 b
			EASTERN WHITE PINE				
Pisolithus as vegetative inoculum	94 a	24.2 a	0.80 a	0.16 a	3.0 a	35 a	6.6 a
Natural inoculum	87 b	14.8 b	0.45 b	0.03 b	0.5 b	8 b	1.4 b

[1] Means in each column followed by the same letter are not significantly different ($P = 0.05$).
[2] Seedling volume (cm³) = (root collar diameter)² × height.
[3] PVI is plot volume index (cm³) = mean seedling volume × No. surviving trees.
[4] PWI is plot weight index (gm) = mean seedling top fresh weight × No. surviving trees.

of 5 and 11 were found in subplots of loblolly and Virginia pines, respectively, after the second season. Basidiocarps of *Thelephora terrestris* occurred abundantly near seedlings of all pine species in the NI treatment. A *Rhizopogon* sp., associated with pure white ectomycorrhizae, was found infrequently near all pine species and treatments. Also, a few *Pisolithus* ectomycorrhizae were found on one loblolly pine seedling from the NI treatment after the second season.

All pine species in all treatments had abundant ectomycorrhizal development of various colors and morphology. Seedlings with abundant *Pisolithus* ectomycorrhizae also had other ectomycorrhizal types. The distinctive black ectomycorrhizae formed by *Cenococcum graniforme* (Sow.) Ferd. and Winge occurred occasionally.

Site 2. Survival and growth of loblolly pine and growth of Virginia pine were significantly improved by *Pisolithus* ectomycorrhizae (Table 2). Fresh weights were approximately 70 percent, and the plot indices were between 50 and 75 percent greater for both pine species from the VMI treatment in comparison to the controls. *Pisolithus* was confirmed on 100 and 87 percent of the loblolly pines, and on 100 and 74 percent of the Virginia pines from the VMI and BI treatments, respectively. On average, 2 basidiocarps of *Pisolithus* were found per subplot of VMI in loblolly pine and 10 in Virginia pine. Two basidiocarps of *Pisolithus* were detected per subplot of loblolly pine from the BI treatment. Numerous basidiocarps of *Thelephora* were found in the NI treatments of both pine species. The unidentified *Rhizopogon* sp. and associated white ectomycorrhizae were observed sporadically. Several other

TABLE 2. Survival and growth of loblolly and Virginia pine seedlings with Pisolithus tinctorius *or natural ectomycorrhizae after 2 years on Site 2 near Morganton, NC. Each value is the mean of 5 replicates each originally containing 20 seedlings.*[1]

Ectomycorrhizae from nursery formed by—	Survival (percent)	Height (cm)	Root collar dia (cm)	Seedling vol. (cm³)[2] / 100	PVI (cm³)[3] / 100	Top fresh wt (gm)	PWI (gm)[4] / 100
LOBLOLLY PINE							
Pisolithus as vegetative inoculum (VMI)	94 ab	87.4 a	1.79 a	2.80 a	52.6 a	408 a	76.7 a
Pisolithus as spore inoculum (BI)	98 a	80.2 ab	1.76 a	2.48 ab	48.6 ab	360 a	70.6 a
Natural inoculum (NI)	90 b	74.3 b	1.61 a	1.93 b	34.7 b	241 b	43.4 b
VIRGINIA PINE							
Pisolithus as vegetative inoculum	94 a	85.9 a	2.12 a	3.86 a	72.6 a	672 a	126.3 a
Natural inoculum	92 a	75.0 b	1.84 b	2.54 b	46.7 b	385 b	70.8 b

[1] Means in each column followed by the same letter are not significantly different ($P = 0.05$).
[2] Seedling volume (cm³) = (root collar diameter)² × height.
[3] PVI is plot volume index (cm³) = mean seedling volume × No. surviving trees.
[4] PWI is plot weight index (gm) = mean seedling top fresh weight × No. surviving trees.

forms of ectomycorrhizae, including that formed by *Cenococcum*, were also associated with both pine species in all treatments.

Site 3. *Pisolithus* ectomycorrhizae improved seedling volumes and plot indices of loblolly pine, and also improved survival, growth, and 3 plot indices of slash and sand pines (Table 3). Seedlings of both loblolly and slash pines from the BI treatments were not different from the control seedlings. Over 1.5 times as many sand pine seedlings survived and had one-third more fresh weight with *Pisolithus* ectomycorrhizae as with natural ectomycorrhizae.

At the end of the first season, all pine seedlings from the VMI treatment and approximately 75 percent of the BI seedlings had abundant *Pisolithus* ectomycorrhizae. Basidiocarps of *Pisolithus* were not observed in subplots at this time. Basidiocarps of *Thelephora* were abundant, however, and those of the unidentified *Rhizopogon* sp. were infrequent on all pine species and treatments.

After the second season, 40 percent of the loblolly pine, 60 percent of the slash pine, and 67 percent of the sand pine from VMI had *Pisolithus* persisting on the roots. *Pisolithus* was detected on 13 percent of the loblolly pine and 33 percent of the slash pine from BI. Three basidiocarps of *Pisolithus* were found per subplot of slash pine from the VMI treatments after the second year, while none were found near seedlings of other treatments. Few *Thelephora* or *Rhizopogon* basidiocarps were observed after the second season. Seedlings in all treatments had abundant ectomycorrhizae of several different morphological types in mixture on their roots. *Cenococcum* ectomycorrhizae occurred only rarely on this site.

Site 4. Loblolly pines with *Pisolithus* ectomycorrhizae had significantly greater seedling volumes, fresh weight, and plot indices than did controls (Table 4). Slash pine from VMI also had significantly greater survival, seedling volumes, fresh weights, PVI, and PWI than those in BI. All slash pines with *Pisolithus* ectomycor-

TABLE 3. Survival and growth of loblolly, slash, and sand pine seedlings with Pisolithus tinctorius *or natural ectomycorrhizae after 2 years on Site 3 near Brooksville, FL. Each value is the mean of 5 replicates each originally containing 20 seedlings.*[1]

Ectomycorrhizae from nursery formed by—	Survival (percent)	Height (cm)	Root collar dia (cm)	Seedling vol. (cm³)[2] / 100	PVI (cm³)[3] / 100	Top fresh wt (gm)	PWI (gm)[4] / 100
LOBLOLLY PINE							
Pisolithus as vegetative inoculum (VMI)	98 a	87.4 a	2.66 a	6.18 a	121.1 a	655 a	130.3 a
Pisolithus as spore inoculum (BI)	90 a	79.5 a	2.43 a	4.69 b	84.4 b	519 a	93.4 b
Natural inoculum (NI)	98 a	78.0 a	2.56 a	5.11 b	100.2 b	564 a	110.5 b
SLASH PINE							
Pisolithus as vegetative inoculum	95 a	101.5 a	3.56 a	12.86 a	244.3 a	1104 a	209.8 a
Pisolithus as spore inoculum	80 b	93.7 a	3.13 b	9.18 b	146.9 b	785 b	125.6 b
Natural inoculum	78 b	93.9 a	3.11 b	9.08 b	141.7 b	824 b	128.5 b
SAND PINE							
Pisolithus as vegetative inoculum	43 a	116.4 a	2.91 a	9.86 a	84.80 a	1358 a	116.8 a
Natural inoculum	16 b	104.7 a	2.63 a	7.24 b	23.17 b	1024 b	32.8 b

[1] Means in each column followed by the same letter are not significantly different ($P = 0.05$).
[2] Seedling volume (cm^3) = (root collar diameter)^2 × height.
[3] PVI is plot volume index (cm^3) = mean seedling volume × No. surviving trees.
[4] PWI is plot weight index (gm) = mean seedling top fresh weight × No. surviving trees.

rhizae had a significantly better PVI than controls. *Pisolithus* ectomycorrhizae significantly increased sand pine survival along with all growth parameters, including plot indices. Nearly five times as much plot volume occurred on sand pine seedlings with *Pisolithus* ectomycorrhizae than with natural ectomycorrhizae. This volume increase resulted from heights and root collar diameter increase, in association with nearly a twofold increase in survival.

After the first growing season, 87 percent of the loblolly and slash pines and all of the sand pines from the VMI treatment had abundant *Pisolithus* ectomycorrhizae. Ninety-three percent of the loblolly and 73 percent of the slash pines from the BI treatment had abundant *Pisolithus.* After the second season, 53 percent of the loblolly and sand pines and 80 percent of the slash pine from the VMI treatment had *Pisolithus* ectomycorrhizae or detectable hyphal strands on their roots. The percentages of loblolly and slash pines from the BI treatment with *Pisolithus* ectomycorrhizae were the same as in the VMI treatment. Seedlings in all treatments had abundant ectomycorrhizae of various colors and morphology on their roots. *Cenococcum* ectomycorrhizae were not observed on this site.

Site 5. In the nonfertilized plots, slash pine seedlings with *Pisolithus* ectomycorrhizae survived better and grew more than twice as fast (i.e., seedling and plot volumes) as seedlings having natural ectomycorrhizae (Table 5). *Pisolithus* ectomycorrhizae did not improve either survival or growth of slash pine in fertilized

TABLE 4. Survival and growth of loblolly, slash, and sand pine seedlings with Pisolithus tinctorius *or natural ectomycorrhizae after 2 years on Site 4 near Brooksville, FL. Each value is the mean of 5 replicates each originally containing 20 seedlings.*[1]

Ectomycorrhizae from nursery formed by—	Survival (percent)	Height (cm)	Root collar dia (cm)	Seedling vol. (cm³)[2] / 100	PVI (cm³)[3] / 100	Top fresh wt (gm)	PWI (gm)[4] / 100
			LOBLOLLY PINE				
Pisolithus as vegetative inoculum (VMI)	96 a	99.4 a	2.37 a	5.58 a	107.1 a	655 a	125.8 a
Pisolithus as spore inoculum (BI)	96 a	92.7 a	2.43 a	5.47 a	105.0 a	655 a	125.8 a
Natural inoculum (NI)	96 a	85.4 a	2.01 a	3.38 b	64.9 b	448 b	86.0 b
			SLASH PINE				
Pisolithus as vegetative inoculum	100 a	111.4 a	3.45 a	13.26 a	265.2 a	1258 a	251.6 a
Pisolithus as spore inoculum	93 b	116.0 a	3.07 a	10.93 b	202.2 b	857 b	158.6 b
Natural inoculum	93 b	97.2 b	2.47 b	7.30 c	135.8 c	831 b	154.6 b
			SAND PINE				
Pisolithus as vegetative inoculum	53 a	107.4 a	2.07 a	4.60 a	48.8 a	587 a	62.2 a
Natural inoculum	27 b	79.4 b	1.41 b	1.58 b	8.5 b	275 b	14.9 b

[1] Means in each column followed by the same letter are not significantly different ($P = 0.05$).
[2] Seedling volume (cm^3) = (root collar diameter)^2 $\times$ height.
[3] PVI is plot volume index (cm^3) = mean seedling volume $\times$ No. surviving trees.
[4] PWI is plot weight index (gm) = mean seedling top fresh weight $\times$ No. surviving trees.

plots. Fertilizer alone, however, dramatically increased growth of slash pine, regardless of initial ectomycorrhizal treatment.

After the first growing season, most slash pine from the VMI had abundant *Pisolithus* ectomycorrhizae in both fertility treatments. The majority of the root systems were heavily colonized, but a few seedlings on very wet microsites had only traces of *Pisolithus* or none at all. Five basidiocarps of *Pisolithus* were observed in the nonfertilized plots and three were found in the fertilized plots planted to seedlings with *P. tinctorius*. Seedlings from the NI treatment in both fertility treatments had only moderate amounts of ectomycorrhizal development of three morphological types, while seedlings on wet microsites had only a few ectomycorrhizae of one morphological type. *Thelephora* basidiocarps were also rare in all plots. Evaluations of ectomycorrhizae were not made on this site after the second season.

DISCUSSION

Assuming that top fresh weight of seedlings is a reliable indicator of seedling growth, it appears that seedling volume and plot volume index (PVI) are also valid parameters. With few exceptions, differences in seedling volume and PVI between ectomycorrhizal treatments were associated with significant differences in top fresh weights and PWI. Neither height nor root collar diameter alone, however, were consistently associated with fresh weight. This is obviously due to the fact that

TABLE 5. Survival and growth of slash pine seedlings with Pisolithus tinctorius *or natural ectomycorrhizae with 90 kg/ha of N and 90 kg/ha of P, or no fertilizer after 2 years on Site 5 near Carrabelle, FL. Each value is the mean of 3 replicates each originally containing 50 seedlings.*[1]

Ectomycorrhizae from nursery formed by—	Fertilizer (kg/ha)	Survival (percent)	Height (cm)	Root collar dia (cm)	Seedling vol. (cm^3)[2] / 100	PVI (cm^3)[3] / 100
Pisolithus as vegetative inoculum (VMI)	NONE	83.3 a	72.4 b	1.70 b	2.09 b	0.88 b
	90 N, 90 P	76.7 ab	150.2 a	3.84 a	22.15 a	84.17 a
Natural inoculum (NI)	NONE	72.7 b	56.0 c	1.26 c	0.89 c	0.32 c
	90 N, 90 P	72.7 b	149.9 a	3.82 a	21.88 a	78.77 a

[1] Means in each column followed by the same letter are not significantly different ($P = 0.05$).
[2] Seedling volume (cm^3) = (root collar diameter)^2 $\times$ height.
[3] PVI is plot volume index (cm^3) = mean seedling volume $\times$ No. surviving trees.

weight measures the density of needles, branches, and the stem, whereas height or root collar diameter alone do not. It was observed in many instances that seedlings of similar heights had as much as 100 percent differences in weight, for height measures only one dimension of growth. A major attribute of PVI is its integration of survival, height, and root collar diameter into a single value which can be obtained without destroying test seedlings. In our opinion, it is a good parameter for assessing the total performance of trees in forestation research.

Pine seedlings with root systems produced in the nursery with *Pisolithus* ectomycorrhizae survived and grew faster on routine reforestation sites than seedlings with ectomycorrhizae formed by *T. terrestris* and other fungi that occurred naturally in the nurseries. The data also indicated that seedlings with the greatest number of *Pisolithus* ectomycorrhizae at planting (formed by mycelial inoculum) grew faster than those with fewer *Pisolithus* ectomycorrhizae (formed by basidiospore inoculum). The advantage of mycelial inoculum was shown previously (Marx and others 1976).

The amount of growth stimulation of seedlings by *P. tinctorius* was related to site conditions. More growth stimulation occurs on the low-quality sites (i.e., sites where control seedlings grew poorly) than on the better quality sites (i.e., sites where control seedlings grew well). For example, loblolly and Virginia pines with *Pisolithus* ectomycorrhizae formed by mycelial inoculum in the nursery had approximately 25 percent greater PVI than the control seedlings on Site 1 (Table 1). On Site 2, a lower quality site, there was over a 50 percent increase in the PVI of both pine species (Table 2). Also, the response of sand pine to *Pisolithus* ectomycorrhizae on Sites 3 and 4 was related to site condition. On Site 3, a better quality site for sand pine, *Pisolithus* ectomycorrhizae stimulated about a 250 percent increase in PVI (Table 3). On Site 4, a lower quality site for sand pine, the stimulation by *Pisolithus* was over 450 percent (Table 4). The growth response of slash pine on Site 5 to fertility also exemplifies this site influence. *Pisolithus* ectomycorrhizae increased the PVI by 175 percent over control slash pines in the nonfertilized plots, but in the fertilized plots effects of *Pisolithus* on PVI were not evident (Table 5). It is obvious that the condition of Site 5 was greatly improved by the addition of N and P fertilizer.

It was unfortunate that there was an insufficient number of white pines for testing on a lower quality site in North Carolina. However, the fact that 1-year-old white pine seedlings with *Pisolithus* ectomycorrhizae on a good site for white pine averaged 24 cm in height and 0.8 cm in collar diameter after 2 years is highly relevant

to artificial regeneration of this species. These seedlings had five times more PVI than seedlings with natural ectomycorrhizae.

The variable growth response of pines to *Pisolithus* ectomycorrhizae on high- and low-quality reforestation sites is probably related to the ability of *Pisolithus* to tolerate adverse soil conditions. This fungus has a broad host range and occurs naturally under a variety of soil conditions around the world (Marx 1977). It is also one of the few ectomycorrhizal fungi which can tolerate extremes of soil conditions, including low pH and high soil temperatures found on coal spoils (Marx 1976). Perhaps this ecological adaptation to poor soil conditions allows *P. tinctorius* a competitive advantage over other ectomycorrhizal fungi for new feeder roots. On the better reforestation sites other species of ectomycorrhizal fungi may be more competitive and aggressive than *Pisolithus*.

Our observations indicate that *P. tinctorius* is persisting on pine seedling roots on all sites in this study. But, other ectomycorrhizal fungi, such as *Thelephora terrestris*, *Cenococcum graniforme*, and an unidentified *Rhizopogon* sp., are also occupying more and more new feeder roots. At high inoculum potential (i.e., VMI), *Pisolithus* forms many ectomycorrhizae on seedlings, persists longer, reproduces better, and stimulates more seedling growth than at low inoculum potential (i.e., BI). Although *Pisolithus* is initially dominant on the root system, with time, several species of fungi become involved in the ectomycorrhizal association. The fungal species most ecologically adapted for that particular tree host and soil condition should eventually dominate the root systems.

Clearly, ectomycorrhizae formed by *Pisolithus tinctorius* can: 1) significantly improve the quality of pine seedlings in nurseries (Marx and others 1976) and in containers (Marx and Barnett 1974); 2) significantly increase the survival and growth of pines outplanted on adverse sites (Marx 1976); and 3) based on this report, significantly increase the survival and growth of southern pines outplanted on routine reforestation sites. *Pisolithus* forms ectomycorrhizae or is associated with over 73 species of forest trees, has been found in over 33 countries and 38 states in the United States (Marx 1977). Therefore, the use of this fungal symbiont as a biological tool for reclamation and reforestation efforts throughout the world appears highly promising.

Literature Cited

Donald, D. G. M. 1975. Mycorrhizal inoculation of pines. South African For J 92:27–29.

Fassi, B., and Anna Fontana. 1969. Mycorrhizal synthesis between *Pinus strobus* (eastern white pine) and *Tuber maculatum*. II. Development of transplanted seedlings and production of ascocarps. Allionia 15:115–120.

Göbl, F. 1974. Mykorrhiza-versuche bei paperpot-samlingen. I. Impfungen von Larche. Centralblatt Gesamte Forstwesen 91: 74–87.

Göbl, F. 1975. Erfahrungen bei der Anzucht von Mykorrhiza-Impfmaterial. Centralblatt Gesamte Forstwesen 92:227–237.

Lamb, R. J., and B. N. Richards. 1974. Inoculation of pines with mycorrhizal fungi in natural soils. I. Effects of density and time of application of inoculum and phosphorus amendment on mycorrhizal infection. Soil Biol Biochem 6:167–171.

Marx, D. H. 1976. Use of specific mycorrhizal fungi on tree roots for forestation of disturbed lands. *In* Proc Conf on Forestation of Disturbed Surface Areas, Birmingham, Alabama, April 14–15, p 47–65. USDA, Forest Service, State and Private Forestry and International Forest Seed Company.

Marx, D. H. 1977. Tree host range and world distribution of the ectomycorrhizal fungus *Pisolithus tinctorius*. Can J Microbiol 23:217–223.

Marx, D. H., and J. P. Barnett. 1974. Mycorrhizae and containerized forest tree seedlings. *In* North Am Containerized Forest Tree Seedling Symp, Denver, Colorado, p 85–92. Great Plains Agric Council Publ 68.

MARX, D. H., and W. C. BRYAN. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. Forest Sci 21:245–254.

MARX, D. H., W. C. BRYAN, and C. E. CORDELL. 1976. Growth and development of pine seedlings in nursery soils infested with the fungal symbiont *Pisolithus tinctorius*. Forest Sci 22:91–100.

MOSER, M. 1958. Die kunstliche Mykorrhizaimpfung von Forstpflanzen. II. Die Torfstreukultur von Mykorrhizapilzen. Forstwiss Zentralbl 77:257–320.

MOSER, M. 1959. Die kunstliche Mykorrhizaimpfung von Forstpflanzen. III. Die Impfmethodic im Forstgarten. Forstwiss Zentralbl 78:193–202.

TAKACS, E. A. 1967. Producion de cultivos puros de hongos microizogenos en el Cento Nacional de Investigaciones Agropecuarias Casten lar. Idia Suppl For 4:83–87.

THEODOROU, C. 1971. Introduction of mycorrhizal fungi into soil by spore inoculation of seed. Aust Forest 35:23–26.

THEODOROU, C., and G. D. BOWEN. 1970. Mycorrhizal responses of radiata pine in experiments with different fungi. Aust Forest 34:183.

VOZZO, J. A., and E. HACSKAYLO. 1971. Inoculation of *Pinus caribaea* with ectomycorrhizal fungi in Puerto Rico. Forest Sci 17:239–245.